Micro-Algal Biotechnology

Subjects: Biotechnology & Applied Microbiology
Contributor: smegnew Birlie

Environment is everything including both the living and non living things in the earth's ecosystem. Today the pollution of the environment is a serious concern facing humanity and other life forms in the world. The environment is daily polluted by natural and anthropogenic (man-mad) factors due to inattention and improper use of the existing science and technology. Several physicochemical methods are used for protection of the pollution in the environment but these techniques have its own advantage and disadvantage. In recent years, using biological methods specially, microalgae for environmental pollution protection is the best one, because it is suitable, produce less and or non toxic products, cost effective and eco-friendly methods to our environment. Microalgae are the important groups of living organisms used for biotechnological utilizations such as for environmental protection, Agricultural usage, biofule production, pharmaceutical production etc. without any environmental factor. This review focuses on the environmental applications of micro-algal biotechnology for treatment of wastewater nutrients, removal of heavy metals from the natural water, mitigation of CO_2, removal of dyes and dyestuffs from their effluents and their agricultural usage without any factor in the natural environment.

Keywords: Microalgae ; Environment ; Pollution ; Protection ; Wastewater.

1. Introduction

Microalgae also called phytoplankton are the first life creature organisms which exist in the Earth's ocean more than 3 billion years ago, when the Earth's environment created. They are microscopic unicellular or simple multi-cellular structure; photosynthetic, prokaryotic or eukaryotic organisms that have chlorophyll and produce oxygen (O_2) by immobilizing carbon dioxide (CO_2) in the atmosphere through photosynthesis and also they can produce carbohydrates, proteins and lipids. Because of their simple morphological structure they can grow rapidly and live in harsh environment. Among prokaryotic microalgae Cyanobacteria (Cyanophyceae) and eukaryotic microalgae green algae (Chlorophyta) and diatoms (Bacillariophyta) are listed as an example. Biologists have categorized microalgae in terms of their pigmentation, life cycle and basic cellular structure in to important classes of microalgae such as diatoms (Bacillariophyceae), the green algae (Chlorophyceae), the golden algae (Chrysophyceae) and the blue–green algae (Cyanophyceae). The minimal nutritional requirement of microalgal biomass is approximately estimated by the molecular formula $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar, 2004; Demirbas, 2010). About 100,000 different types of microalgae living in the oceans and in fresh water (lakes, ponds, and rivers). The main requirements for their growth and survival are sunlight, water, nutrients and arable land. Therefore; reproductive distribution of microalgae is mostly determined by these environmental factors. Through their diverse modes of nutrition (phototrophy, heterotrophy, mixotrophy), microalgae can efficiently remove a wide range of chemicals from aqueous matrices. Recently, microalgae are the important groups of living organisms used for biotechnological utilizations such as for environmental protections (for wastewater treatment and used as biological tool for assessment of environmental toxicants), for production of foods, pharmaceutical applications, bioactive compound production, cosmetics production, agricultures and biofuels usage (Spolaore *et al.,* 2006, Marques *et al.,* 2011). The use of microalgae in biotechnology is the best solution for environmental protection, however small size of single cells are difficult in the harvesting of microalgae after treating the wastewater in the application of biotechnological processes to those organisms. Therefore, cell immobilization techniques have been developed in order to solve those problems (Jimnez-Perez *et al.,* 2004). The commonly used polymers for the microalgae immobilization are calcium alginate, carrageenan and chitosan. Among this immobilized microalgae beads are easy to harvest and reuse. De-Bashan and Bashan (2010) reviewed that an immobilized cell is defined as a cell that by natural or artificial means is prevented from moving autonomously of its neighbors to all parts of the aqueous phase of the system under study. Therefore, using immobilized microalgae are effective method for environmental protection. Environment is everything or the sum total of our natural world that can be including both the living and non living things in the earth's ecosystem. These environments are always polluted by different types of pollution such as water, air, soil, thermal, noise, radioactive, land, industrial, marine, personal, light, etc pollutions which can release physical and chemical wastes that causes harmful effects on the surrounding environments. Today on our planet most serious problems facing humanity and other life forms due to environmental pollution (the contamination of the physical and biological components of the earth/atmosphere system). Environmental pollution, the large volume of compost and non-degradable waste materials, heavy air pollution, continual destruction of the ozone layer, and severe climate change are all due to inattention and improper use of the existing science and technology. There are many causes for environmental contaminations. Among these causes environment ecosystem and natural water bodies are contaminated with wastewater nutrients () which leads to eutrophication, toxic chemicals from agricultural and pharmaceutical products and toxic gases from various industries. This wastewater are comes from textile, pharmaceutical, paper, beverage, horticultural, etc. industries. These contaminants not just can reach into our drinking water supplies but endanger aquatic ecosystems and other organisms. Therefore suitable treatment of the wastewater nutrients before discharging into water bodies is required. To remove these contaminants from wastewater the recent technologies used are expensive, produces highly sludge content and harsh to the nature. To avoid the negative impacts caused through the removal of contaminants, environmentally friendly and sustainable methods should be applied. Microalgae are the alternative biological treatment methods to remove nutrients (Mallick, 2002). But there is a limitation to develop wastewater treatment systems based on microalgae during harvesting process of their biomass at the final of treatment process. To solve this problem immobilization of cells are the best choice mechanism that used to increase the cells retention time within the bioreactors and higher metabolic activity (Tam *et al.,* 1994).

The other causes for contamination of the natural waters are dyes and dyestuffs from wide range of textile, leather, plastic, paper, paint, food, pharmaceutical, cosmetics and other significant manufacturing industries. The purposes of dyes are to produce different color combinations to meet the demands of fashion, to produce brightness and other. These dyes have different structural and chemical properties i.e. acidic, basic, reactive, azo, diazo, disperse, and others. Annually more than 100,000 commercially available dyes and nearly 1 million tons of dyestuffs are produced worldwide (Garg and Tripathi, 2017). The textile industry is an important and the major industrial economic sector in Ethiopia, contributing total earnings of ETB 990.0 million (US$ 49 million) from manufactured exports in 2014 (Ethiopian Textile Industry Development Institute (ETIDI, 2016)). The different process stages of textile industry uses a large amount of water for dyeing, printing, and bleaching, which producing a large quantity of wastewater. Therefore; every year high amount of polluted effluents are discharged from textile processing which consumes large amounts of water. These effluents from textile industries are characterized by high salinity, temperature and chemical oxygen demand (COD) and strong color (Mantzavinos and Psillakis, 2004). As a result removal of textile dyes from the effluent before its disposal in the water bodies is extremely important. Although several physical and chemical methods have been developed for removal of dye from textile effluent, these methods are not suitable due to the production of large amounts of toxic sludge, aromatic amines, and other secondary waste products. Because of their sustainability to our environment, cost effectiveness, eco friendly nature, and produces less toxic and/or non-toxic compounds; numerous biotechnological approaches by using microalgae have been recommended to overcome the problem of physiochemical treatment methods using microorganisms for the treatment of textile dyes and industry effluent as microorganisms play vital roles in the mineralization of xenobiotic compounds (Kurade *et al.,* 2012).

The emissions of CO_2 are the other causes for the environmental contamination and the worlds concern. The increment of concentrations of greenhouse gases in the world's atmosphere is leading fear to a greater extent. Among the greenhouse gases CO_2 is the main one and it was released 28.8 gigatonnes into the atmosphere in 2007 alone (International Energy Agency, 2009) and the IEA also predicted that this number would be reach to 34.5, 40 billion tons by the year 2020 and 2030 respectively, an average rate of growth is 1.5% per year. Agricultural synthetic fertilizers and heavy metals are the other causes of environmental pollutions.

The article of this review focuses on the environmental applications of microalgae for the treatment of wastewater nutrients, removal of dyes and dyestuffs from their effluents, mitigation of CO_2, removal of heavy metals from the natural water, and their agricultural usage without any factor in the environment. Using micro-algal processes for the removal of contaminants is still not widely used and adequately studied in Ethiopia. In Ethiopia there is no known documented micro-algal distribution and resources for biotechnological applications until this time. The aim of this paper is to address the various biotechnological applications of microalgae in the environment and to offer attention for micro-algal biotechnology researches in Ethiopia.

2. Application Micro algal biotechnology for removing pollutants from polluted environments

2.1 Application of Micro algal biotechnology for Wastewater treatment

As we know water is the most basic and essential compound of all living processes in our water planet and it should be treated before usage. Wastewater is the water with poor quality that contains more amounts of pollutants and microbes. Wastewater contains different types of materials like soluble organic, inorganic, insoluble inorganic materials, macro solids, toxins, etc. and the soluble parts of waste by-processing like carbon, nitrogen and phosphate which are used for the microalgae cultivation. The discharge of wastewater containing various contaminants into the aquatic ecosystems or water bodies is a major concern for our environment and to public health. These contaminated wastewater compounds may be cause physical, chemical, biological, and environmental impacts in aquatic habitat, biodiversity and water quality (Aleem, 2003). Wastewater treatment is the significant measure to reduce the pollutant and other contaminants present in wastewater. Rawat *et al*. (2010) reviewed that there are three treatment methods of wastewater these are; the primary treatment method which is the first step in wastewater treatment method that removes the solids, oil, and grease from wastewater. Secondary treatment or biological treatment method is the second step, which abuses microorganisms to eradicate the chemicals present in wastewater. The tertiary treatment method is the final step; which eradicates the microbes from wastewater before discharging into the river. Although many attempts to stop the destruction and pollution of water resources, it requires an attention on the necessity of the use of new methods to prevent contamination of water resources (Delplanque, 2013). Microalgae wastewater treatment is eco-friendly and offers cost effective way of nutrient removal, biomass production and have numerous applications in environmental biotechnology, mainly for bioremediation, bioassay and biomonitoring of wastewaters and toxicants (Mulbry *et al.,* 2008). The high rate algae pond (HRAP) system has been shown to be an effective system to treat wastewater (Hoffmann, 1998; Phang *et al.,* 2001). The system consists of shallow pond mixed by paddle-wheels to improve nutrient transfer and photosynthetic efficiency to optimize algal growth. The HRAP system is particularly suitable for tropical climate with the warm weather and abundance of sun light throughout the year. HRAP used for efficient reduction of pollutants such as chemical oxygen demand (COD), biological oxygen demand (BOD), nutrients (nitrogen and phosphorus). Microalgae grown in HRAP were advantageous in treating various wastewaters such as rubber effluent, palm oil mill effluent (POME) and municipal wastewater (Garcia *et al.,* 2000; Phang *et al.,* 2001). *Spirulina platensis* grown in HRAP have been used to achieve to reduced 98.0%, 99.9% and 99.4% chemical oxygen demand, ammoniacal-nitrogen and phosphate respectively from anaerobically digested starch factory (Phang *et al.,* 2000). In certain studies combination of numerous microalge has high efficiency in wastewater treatment. For example a group of five microalgae, *Chlorella vulgaris, Scenedesmus quadricauda, Euglena gracilis, Ankistrodesmus convolutus* and *Chlorococcum oviforme* grown in HRAP was successfully used to treat landfill leachate (Mustafa *et al.,* 2012). In other studies dual uses of Chlorella sorokiniana and Scenedesmus obliquus cultivated with in filtered raw sewage were used for biomass production and wastewater treatment without the requirement of any additional treatment. In this study S. obliquus showed greater potential for removing organic carbon (76.13 ± 1.59% COD removal), nutrients (98.54 ± 3.30% N-removal, 97.99 ± 3.59% P-removal) and comparable pathogens removal (99.93 ± 0.12% total coliforms removal, 100% faecal coliform removal) in comparison to C. sorokiniana (69.38 ± 1.81% COD removal), (86.93 ± 3.49% N-removal, 68.24 ± 11.69% P-removal), (99.78 ± 0.12% total coliforms removal, 100% faecal coliform removal) in 15 days(Gupta *et al.,* 2016). Mostafa *et al.* (2012) reported that the nine microalgae species of (green and blue green microalgae) *Chlorella vulgaris, Spirulina platensis*, *Nostoc humifusum Nostoc muscorum, Anabeana flous aquae, Anabeana oryzae, Phormedium fragile* W*ollea saccata,* and *Oscillatoria* sp., on domestic wastewater are effective in nutrient removal and also using the domestic wastewater as nutrient media for microalgae cultivation is appropriate and low-cost compared to the conventional cultivation methods. In the semicontinuose operating mode immobilized *Chlorella vulgaris* and *Scenedesmus obliquus* were effective in removing phosphorus and nitrogen from urban wastewater (Ruiz-Marin *et al.,* 2010). Other study shows that, co-immobilisation of microalgae and bacteria have confirmed to be more efficient in removing nutrients for instance nitrate, ammonium and phosphate from wastewater more willingly than immobilised microalgae lacking bacteria (De-Bashan *et al.,* 2002). Zhang *et al.* (2008 & 2012) also reported that Immobilized *Scendesmus sp.* and *Chlorella sp*. isolated from municipal wastewater, entrapped in calcium alginate as algal sheets was employed to remove inorganic nutrients (NH (4) (+)-N and PO4 (3-)-P) and the removal efficiency for Scendesmus sp. was 100% after 135 min and 15 min respectively and for Chlorella sp. was 98.81% and 100% respectively after 4 h in domestic secondary effluents, from artificial and real domestic secondary effluents in parallel-plate bioreactor after starvation. These two immobilized microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*) were also used to remove phosphate, ammonium and nitrate from wastewater to less than 10% of their initial concentration within 9 days in the twin-layer photobioreactor system (Shi *et al.,* 2007). The investigation indicates that using this twin-layer system technology for algal cell immobilization has 100% cell immobilization than the other methods, because the method can successfully separate microalgae from the bulk of their growth medium. The twin-layer system is the new technology for cell immobilization technique that used for removal of nutrients from wastewater and an effective means to reduce nitrogen and phosphorus levels in wastewater. *Chlorella vulgaris* were also effective for bioremediation in removing NH (4)-N (44.4-45.1%), PO (4)-P (33.1-33.3%) and COD (38.3-62.3%) from textile wastewater (Lim *et al.,* 2010). The microalgae *Chlorella sp*. has the removal efficiency in the percentage of 90.8, 80.1, 98.9, 87.6, and 90 % of the waste nutrients BOD, COD, NH4-N, NO3–N and PO4–P respectively (Hammouda *et al.,* 2015).

2.2 Application of micro algal biotechnology for Removal of heavy metals

The major environmental concern of water bodies are contamination by heavy metals because these contaminants are very toxic, xenobiotic and or recalcitrant and exhibit a bioaccumulation tendency (Pacheco *et al.,* 2015a). Metals such as sodium, magnesium, calcium, nitrogen, potassium, iron, copper, manganese, molybdenum, vanadium, manganese, cobalt, zinc and nickel are vital for biological functions that are directly or indirectly involved in all phases of microbial growth. Whereas; heavy metals like mercury, cadmium, arsenic, antimony, chromium, silver, gold and lead are not well-known to have essential biological functions in microbial growth. Among the 19 heavy metals lead, cadmium, and mercury are known to be extremely toxic. Heavy metals constitute a diverse group of elements widely varied in their chemical properties and biological functions. Heavy metals are environmental pollutants results from Anthropogenic activities (mining, smelting operation and agriculture) as well as natural activities which locally increased the levels of heavy metals such as Cd, Co, Cr, Pd, As and Ni in soil up to dangerous levels. Although the presence of these heavy metal ions are used in industrial, pharmaceutical and agricultural processes; they are highly toxic and carcinogenic to human beings, alter biochemical cycles of living bodies, serious damage to the aquatic life and causes teratogenicity changes in plants, animals and humans (Shanab *et al.,* 2012). They are also integrated into the food chain and accumulate along the different tropic levels producing harmful effects, especially to consumer organisms of the top of these chains. Heavy metals contamination is a serious issue of concern around the world due to the increase in the use and processing of heavy metals during various activities to meet the needs of the rapidly growing population. Soil, water and air are the major environmental compartments which are affected by heavy metals pollution. Using immobilized microalgae in biotechnology is the solution to reduce the contamination of heavy metals. For instance, most of immobilized *Chlorella sp.* in alginate beads was useful in removing heavy metals like Cu^{2+}, Ni^{2+} and Cd^{2+} ions in 25mg/L initial concentration of each metal 97.1%, 50.94%, 64.61% removal and 150.07, 48.87, 101.73mg/g biosorbent, respectively at PH 5 for 180 min from drinking water (Petrovic and Simonic, 2016). And also immobilized *Chlorella sp.* in alginate beads was useful to remove 98% chromium (VI) in the concentration of 75mg/L at PH 3 for 9 days from synthetic waste water (Rao *et al.,* 2013) and consistently (>90%) removal and (about 100%) recovery of Lead were achieved in the concentration of 50mg/L at PH 6 for subsequent cycles (Hameed, 2006). Additionally immobilized *Chlorella sp.* in alginate beads was useful to remove 59.67% Cadmium in the concentration of 20 ppm for 60 min in aqueous solution (Valdez *et al.,* 2018). Akhtar *et al.* (2003) also investigated that free and immobilized *Chlorella sorokiniana* removed cadmium from 10 mgl(-1) solution at the efficiency of 92.7% and 97.9% respectively. The immobilized *Anabaena sp.* system has been investigated as a potential biosorbent for the removal of 80 % cadmium from aqueous solutions and the full adsorption time taking place 50 min (Clares *et al*., 2015). Microalgae are used in bioassay of organisms to examine the toxicity of harmful pollutants for instance heavy metals, pesticides and pharmaceuticals.The three fresh water microalgae *Phormidium ambiguum, Pseudochlorococcum typicum* and *Scenedesmus quadricaudavar quadrispina* could be used for removal and tolerant of mercury (Hg^{2+}), lead (Pb^{2+}) and cadmium (Cd^{2+}) in aqueous solutions as a single metal species at concentration of 5-100mg / L under controlled laboratory conditions and among the three *sp., P. typicum* from aqueous solution showed that the highest percentage of metal bioremoval occurred in the first 30 min of contact recording 97% (Hg^{2+}), 86% (Cd^{2+}) and 70% (Pb^{2+}) respectively (Shanab *et al.,* 2012). The cells of *Oscillatoria limosa* was used for biosorbation of the three metals (10.8 mg/g biosorbent in 40 mg/L concentration Cd, 17.2 mg/g biosorbent in 80 mg/L concentration Hg and 16.4 mg/g biosorbent in 60 mg/L concentration Pb) and their order of removal was found to be 82, 78 and 72% at 120 min respectively (Sivakami *et al.,* 2015). Fekry *et al.* (2018) also reported the microalgae strains namely *Anabaena flos aquae, Nostoc elepsosporum, Nostoc linkia, Anabaena variabilis* and *Chlorella vulgaris*, were effectively remove the heavy metals Chromium, lead, iron, copper, Molybdenum and arsenic from the textile wastewater effluent after 4 weeks incubation periods under continuous illumination. Hammouda *et al*. (2015) also reported *Chlorella sp*. have the ability to remove heavy metals in the percentage of nickel (99.5 %), Mn (73.2 %), Fe (92.2 %), Cu (54.5 %), Zn (51.4), Cr (56.3 %), Mo (99.7 %), Al (98.8 %), Si (48.5 %), V (100 %), Ti (100 %), Sr (41.9 %).

2.3 Application of micro algal biotechnology for CO_2 sequestration

In the last 50 years the emissions of CO_2 have been raised radically and still continue at the rate of 3% annual increase. A long time increase of the earth's atmospheric temperature or global warming is caused by the emission of greenhouse gases. In the earth's atmosphere the primary greenhouse gases (GHG) are Carbon dioxide (CO_2), Methane (CH4), Nitrous oxide (N_2O), Hydro fluorocarbons (HFCs) and per fluorocarbons (PFCs), these are human origin gases that contribute up to 95% of the total to boost in global warming. Although the durability of these gases varies in the atmosphere; it's very high at all times i.e. 80% lasts for 200 years and the other 20% can take up to 30,000 years to vanish. Among these gases CO_2 is in charge for over 60% of the level of global warming as the result of fuel use, deforestation, production of cement and other causes (Huang and Tan, 2014). Fossil fuels, together with oil, coal and natural gas, are providing about 85 % of energy globally. The requirement of energy is increasing continuously because of increase in industrialization and population. Continuous uses of fossil fuels and their combustion leads to emission of greenhouse gases like carbon dioxide, nitrogen oxides, methane, sulfur dioxide and volatile organic compounds (Gavrilescu and Chisti, 2005; Verma *et al.,* 2010). Combustion of fossil fuels emits harmful gases into the atmosphere and 73% of the CO_2 in the atmosphere comes from burning of these fossil fuels (Verma *et al.,* 2010). By means of rapidly rising world energy demand and high fossil fuel prices, a forceful interest is being focused on the photosynthetic organisms which can store lipids in the form of triacylglycerides (TAGs), produces renewable energy that can be the best alternative to limited oil resources, it is environment-friendly nature and also it shares a total of low GHG emissions compared with fossil fuels. Although various CO_2 capture technologies have been proposed, photosynthetic microalgae can be the greatest substitute for fossil fuels due to its higher photosynthetic efficiency, its environment friendly nature, use of unproductive lands, exhausting non-renewable reserves and its ability to grow on wastewater by using broad variety of nutrient. Microalgae are most suitable in mitigation of climate change because it reduces the emission of fuel gases and have fast growing capability due to the consumption of CO_2 and having more oil content than any other source. Microalgae are also able to produce biomass (which resulting the releases of oxygen (O_2) into the atmosphere) from solar energy, CO_2 and nutrients in the water bodies. Currently these biomasses are the alternative micro algal based sources of energy for production of various types of Biofuels for example; Biodiesel (Chisti, 2007; Craggs *et al.,* 2011; Kumar *et al.,* 2013), Biodiesel production from W*ollea saccata, Anabeana flous aquae, Oscillatoria* sp. *Anabeana oryzae, Spirulina platensis*, *Chlorella vulgaris, Phormedium fragile, Nostoc humifusum* and *Nostoc muscorum* (3.9, 4.0, 4.3, 4.5 ,7.8, 8.8 , 10.1, 10.2 and 12.52 % respectively) in the synthetic media and *Oscillatoria* sp., W*ollea saccata, Anabeana oryzae, Spirulina platensis*, *Anabeana flous aquae, Nostoc muscorum, Phormedium fragile, Chlorella vulgaris* and *Nostoc humifusum (*3.8, 4.0 4.7, 5.0, 5.0, 7.4, 8.4, 8.5, and11.8% respectively) in the domestic wastewater (Mostafa *et al.,* 2012), Bioethanol production from *Scenedesmus dimorphus* under aeration of carbon dioxide enriched air (Chng *et al.,* 2017) and from *Arthrospira* (Spirulina) *platensis* by Carbohydrate-Enriched biomass (Markou *et al.,* 2013), Biogase production from *Chlorella vulgaris* and *Chaetoceros* sp. by microalgae fermentation respectively (Perazzoli *et al.,* 2013, Negara *et al.,* 2017), Bio-hydrogen production from the wild-type of *Chlorella sorokiniana* a green microalge produced hydrogen in photobioreactor coupled to a small Proton Exchange Membrane Fuel Cell (PEMFC) (chader *et al.,* 2011). Recently, cultivation of microalgae has established new consideration on account of their utility as an achievable CO_2 sequestration technology (Ono and Cuello, 2006; Hsueh *et al.,* 2007; Jacob-Lopes *et al.,* 2008; Verma *et al.,* 2010). A number of the green microalgae genera such as *Dunaliella, Chlorella, Euglena, Botryococcus, Scenedesmus*, and *Chlorococcum* are the effective carbon sequesters (Bhola *et al.,* 2014). Also several studies have been carried out from time to time for the determination of micro algal ability to hold up the high CO_2 concentrations. Microalgae are minute that has to some extent greater photosynthetic efficiency other than plants and they are sunlight driven biochemical factories and very effective CO_2 fixers (Verma *et al.,* 2010). Some species of microalgae able to biodegrade the chemical groups of polycyclic aromatic hydrocarbons (PAHs) other constituents of crude oil and refined petroleum products. For instance, microalgae *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda* and *Raphidocolis captricornutum* on fluoranthene (1.0 mg l^{-1}), pyrene (1.0 mg l^{-1}), and a mixture of fluoranthene and pyrene (each at a concentration of 0.5 mg l^{-1}) within 7 days of treatment are used to degrade PAHs (Lei *et al.,* 2007). Hannon *et al*. (2010) reported that about 40% of the primary productivity O_2 genesis and CO_2 sequestration is achieved by photosynthetic microalgae. So, microalgae play a vital role in the biomitigation of CO_2 as the main cause of global warming, attenuation of greenhouse gas effect, biofuel and valuable secondary metabolite production and carbon balance worldwide.

2.4 Agricultural uses of micro algal biotechnology in the environment

Agriculture was accomplished in the world for a long period of time by human beings without the use of any synthetic fertilizers. But, after many years usage the soil fertility was gradually diminished and agricultural productivity was decline worldwide. Therefore, because of the increment of world's population growth; unbalance demand and supply was happened, in order to solve this problem for the last 50 years farmers become mostly depend on man-made chemical fertilizers and pesticides to improve crop yield and to increase productivity. The present concern is those chemically active fertilizers or pesticides, because using synthetic chemical fertilizers and pesticides causes to various forms of environmental degradation i.e. pollution of air and water; soil depletion; diminishing biodiversity; polluting soil, water and air; and damaging both the environment and human health. Hence, to reduce environmental pollution, to maintain soil fertility, to increase crop productivity and quality, to reduces the amount of the use of chemical fertilizers and pesticides use of alternative source of nitrogen fertilizer is important. The use of microbial products, functional bio-fertilizers and bio-controllers is the crucial alternative and effective ways of methods to control the environmental pollution, to recover soil fertility (soil reclamation) to improve crop production and nutritional quality, biocontrolling of agricultural pests, formation of microbiological crust, agricultural wastewater treatment, recycling of treated water and to substituting costly inorganic nitrogen fertilizer in agriculture. Among these biofertilizers microalgae species are an optional and economically attractive to be used as organic and cheap source of N2, which does not cause soil and water pollution instead of using the expensive and environmental polluted industrial chemical fertilizers. Today Nitrogen-fixing forms of microalgae act as biological or natural fertilizers for rice and other crops. For instance, rice cultivation inoculated with marine microalgae, *Chlorella vulgaris* and *Spirulina platensis* can be used as biofertilizer to enhance rice yield up to 7–20.9% respectively (Dineshkumar *et al.,* 2018). Other study investigates that the use of microalgae polysaccharides as biostimulant of plant growth applied to tomato (*Solanum lycopersium)* and pepper *(Capsicum annum)* plants at different growth stages by spraying the total polysaccharides extract (TPE) from *Spirulina platensis* was increased the plants sizes, roots and number of nodes per plants (Elarroussi *et al.,* 2016). Cellular extracts and dry biomass of the green alga *Acutodesmus dimorphus* culture, applied as a biostimulant, foliar spray, and biofertilizer, respectively, were able to activate faster germination, enhance plant growth and fruit production in tomato plants (Garcia-Gonzalez and Sommerfeld, 2018). In another research findings; the use of cyanobacterial as a biofertilizer should be suggested as an alternative source of inorganic N-fertilizer to increase yield and nutritional quality of kale crop for medium and small-sized farms in the study area (Eshetu *et al.,* 2018). Cyanobacteria also known as blue–green algae because of the presence of phycocyanin and phycoerythrin which frequently cover the chlorophyll pigmentation are the primeval group of prokaryotic microorganisms around for nearly 3.5 billion years and exhibiting the general characteristics of gram-negative bacteria and they are considered as one of the sole and important primary producers in the biosphere, as these prokaryotes possess chlorophyll *a* and conduct oxygenic photosynthesis contributing a majority of the carbon fixation on earth, turning greenhouse gases into carbohydrates and lipids (Bisth *et al.,* 2015). Cyanobacteria have an important role as a natural biofertilizer to maintain and increase soil fertility, as a result increasing crop growth and also they are the major components of the nitrogen fixing biomass. In addition, cyanobacteria as biofertilizer have the advantages of lower cost and reduced production of greenhouse gasses such as, nitrous oxide and carbon dioxide by 30% which results in less pollution of the environment (Eshetu *et al.,* 2018). Cyanobacteria also used as biocontrol agents. In the currently agricultural practices, the conventional pest control methods have not been efficient and chemical pesticide agents inhibit the growth and development of crop plants (Manjunath *et al*., 2010). To solve this problem cyanobacteria play a major role as opposing agents against several pathogenic microbes, insects and weeds (Prasanna *et al*., 2013a). For instance, Natarajan *et al*. (2012) has identified two novel fungicidal compounds as benzoic acid derivative and majuscule amide C from *Calothrix elenkinii* and *Anabaena laxa* respectively. In other finding compounds Chitosanase homologues and micro cystin which have the fungicidal activity were identified from *Anabaena laxa, A. iyengarii* and *A. fertilissima* (Gupta *et al*., 2012). Microalgae also have the ability to stimulate growth activity as plant growth promoters. For example, the microalgae genera *Chlorella, Scenedesmus* and *Chlamydomonas* were tested and show phyto-stimulation and phyto-toxicity activity in cucumber seeds (Patil *et al.,* 2015). The recent findings established that cyanobacteria improved the growth of rice, wheat, maize and soybean respectively grown in pot experiments (Nanjappan *et al.,* 2007 and Sholkamy *et al.,* 2012). Results of these study showed that shoot length, root length, leaf area, plant weight and the number of legumes increased significantly with the addition of combined nitrogen compared to plants not fertilized with nitrogen. Other study shows that long-term usage of synthetic chemicals leads contamination of the soil. For instance, long-term use of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) to control arthropod disease-vectors and agricultural pests causes contamination on soil biological properties which leads to loss of soil fertility (Megharaj *et al.,* 2000). This study suggests that organisms especially microalgae are used as bioindicators. As we know chemical assays are only used to determine the contents of the pollutants; but bioassays can really assess the biological effects on living organisms. Because of their sensitivity to the presence of toxic chemicals, microalgae have the ability to estimate the ecotoxicity, genotoxicity and environmental risk of pollutants both in soil and sediments. Hence, microalgae are often included in the bioassays of environmental pollutants. The common microalgae used for bioassays of toxicants include *Pseudokirchneriella subcapitata*, *Dunaiella tertiolecta*, *Isochrysis galbana*, *Chlorela* sp. (Vannini *et al.,* 2011). Discharge of extra nutrients from fertilizers and industrial wastewater to the aquatic environment can activate overgrowth of microalgae that causes eutrophication (Smith, 2003). Microalgae namely, *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Ankistrodesmus convolutus* were found to be useful for the assessment of nitrogen and phosphorus enrichment in freshwater ecosystems (Chu *et al.,* 2009). Therefore; the use of microalgae as bio-fertilizer was used to reduce the polluted synthetic fertilizers use, used for estimation of environmental pollutants due to their bioindicator activity, maintaining soil fertility and or production of oxygen, and to decide the potentiality of bio-fertilizer application in order to have maximum yield as well as for ensuring eco-friendly environment by avoiding chemical pollution.

2.5 Application of Micro algal biotechnology in Dyes and dyestuffs removal

A dye is a colorant substance that chemically bond to the substrate to which it is being applied in aqueous solution and dyestuff is the derived term of dye that is any soluble pigment. Due to their various structure dyes are difficult to classify, however; they are generally classified into two groups and classes depending on their source, general dye structure and the fiber type as follows: 1) Natural dyes: natural dyes are dyes that are produced from the natural plants for instance; Madder and from animals for example Tyrian purple (sea snails). 2) Synthetic dyes: dyes that are artificially produced from living organisms. Synthetic dyes are also divided into azo dyes and non azo dyes. Azo dyes contains basic, acidic, vat, sulfur, disperse dyes and reactive (based on chemical constitution for instance; chloro-triazine dye, vinyl-sulpone dye, mixed dye etc. and based on dye application for example; cold Brand dye, medium brand dye, high brand dye) (Ajmal *et* *al.,* 2014). Generally dyes, pigments and dyestuffs are important in textile, paper, paint, plastic, food, cosmetics, pharmaceutical and other industries for coloration of paper, cotton, leather, wool, silk, nylon; used as pH indicator, painting multiple products, light-reflecting signs, heat and ultraviolet light (UV) resistant etc. Despite of, their applications for different industry processes, dye products are physically horrible nature and toxicity in the environment. Continuously increasing vast production rate of dyes due to increasing industrialization and population growth have led to the obligation of effective treatment of their wastes. Therefore, a wide range of technologies has been developed for the removal of these apparent and challenging dye wastes to reduce environmental impacts (Ajmal *et al.,* 2014). Among these technologies physical techniques such as; activated carbon, adsorption, reverse osmosis, ultra filtration can be used for dye removal however, these process causing secondary pollution. Chemical techniques such as; chlorination, ozonation, adsorption on organic or inorganic matrices, precipitation, chemical oxidation processes, advanced oxidation processes (Fenton and photo-Fenton catalytic reactions, H_2O_2/UV processes and photo degradation through photo catalysis) are also can be used for dye removal, however; toxic unstable metabolites as a result of most of these processes imparts adverse effects on animal and human health. Biological techniques involving microbiological or enzymatic decomposition and biodegradation under aerobic and anaerobic condition have also been used for dye removal from wastewaters however; the process results incomplete mineralization of toxic and carcinogenic by-products. Hence, the above mentioned techniques have their own draw-backs. Due to their considerable hazard, several authors have attempted to find new forms of treatment to reduce the serious environmental risks caused by various dye effluents. To overcome the above treatment techniques problem numerous biotechnological approaches by using microalgae have been recommended because these methods are sustainable to our environment, cost effective, eco friendly nature, and produces less toxic compounds. El-Sheekh *et al*. (2009) investigated that some algae's from different categories such as *Chlorella vulgaris, Lyngbya lagerlerimi, Nostoc lincki, Oscillatoria rubescens, Elkatothrix viridis* and *Volvox aureus* have the ability to decolorize and remove methyl red, orange II, G-Red (FN-3G), basic cationic, and basic fuchsin dyes from ~4 to 95% according to the algal species, its growth state and the dye molecular structure. Over 90% tributyltin (TBT) could effectively remove at different contamination level for six consecutive cycles by biosorption and biodegradation (Luan *et al.,* 2006) and effective in removing 44.0 % colour at initial concentration of 7.25mg/L from textile dyes and textile factory wastewater (Chu *et al.,* 2009). In another study the five microalgae strains that is *Nostoc elepsosporum, Chlorella vulgaris, Anabaena variabilis, Nostoc linkia* and *Anabaena* were evaluated for their efficiency to remove the red color of the textile wastewater effluent after 4 weeks incubation under continuous illumination. In this study the percentages of these microalgae color removal were 100, 96.16, 88.71, 79.03 and 50.81 % respectively at 600 nm absorbance (Fekry *et al.,* 2018). Other study shows that in the high rate algae pond (HRAP) system using *Chlorella vulgaris* was a good system for final polishing of textile wastewater before discharge, mainly for removal of colour from 41.8% to 50.0% in a range of 0.17 to 2.26 mg chlorophyll a/L (Lim *et al.,* 2010).

3. Conclusion

As conclusion using microalgae technologies system have become many applications in our life and world's environment especially for developing country like Ethiopia. Microalgae have unicellular or simple multi-cellular cell structures and are able to survive and reproduce in almost all environments, that can tolerating a variety of environmental conditions and contaminants. Because microalgae's require only water, light, nutrient and CO2 for their growth and they are fast growing, sustainable to our environment, cost effective, and produces less toxic compounds using microalgae for environment application has multipurpose role. In the future using these micro algal technologies is the solutions for our environmental problems and changes. [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69][70][71][72][73][74][75][76][77][78]

Acknowledgments

Above all I thanks and praise to the almighty God who provide to me healthy, strength, stability and patience to bring this work from the beginning to the end for my initial manuscript. Secondly the author is grateful to Mr, Amare Genetu and Mr. Adugnaw Admas for their useful comments on the manuscript.

References

1. Ajmal, A., Majeed, I., Malik, R.N., Idriss, H., & Nadeem, M.A. (2014). Principles and mechanisms of photocatalytic dye degradation on TiO2 based photocatalysts: a comparative overview. RSC Advances, 4, 37003–37026.
2. Akhtar, N., Saeed, A., & Iqbal, M. (2003). Chlorella sorokiniana immobilized on the biomatrix of vegetable sponge of Luffa cylindrica: a new system to remove cadmium from contaminated aqueous medium. Bioresource Technology, 88, 163– 165.

3. Aleem, A., Isar, J., and Malik, A. (2003). Impact of long term application of industrial wastewater on the emergence of resistance traits in Azotobacter chroococcum isolated from rhizospheric soil. Bioresource Technology, 86(1), 7-13.
4. Bhola, V., Swalaha, F., Kumar, R.R., Singh, M., & Bux, F. (2014). Overview of the potential of microalgae for CO2 sequestration. International Journal of Environmental Science and Technolog, 11, 2103–2118.
5. Chader, S., Chetehouna, K., Mahmah, B., Amrouche, K., & Abdeladim, K. (2011). Biohydrogen production using green microalgae as an approach to operate a small Proton Exchange Membrane Fuel Cell. International Journal of Hydrogen Energy, Elsevier, 36, pp.4089-4093.
6. Chisti, Y. (2007). Biodiesel from Algae. Biotechnology Advances, 25, 294-306.
7. Chng, L. M., Lee, K.T., & Chan, D. C .J. (2017). Evaluation on Microalgae Biomass for Bioethanol Production. IOP Conference Series: Material Science and Engineering, 206, 012018.
8. Chu, W.L., See, T.C., & Phang, S.M. (2009). Use of immobilized Chlorella vulgaris for the removal of colour from textile dyes. Journal of Applied Phycology, 21(6), 641-64.
9. Clares, M. E., Guerrero, M. G., & Garcı´a-Gonza´lez, M. (2015). Cadmium removal by Anabaena sp. ATCC 33047 immobilized in polyurethane foam. International Journal of Environmental Science and Technology, 12, 1793–1798.
10. Craggs, R.J., Heubeck, S., Lundquist, T.J., & Benemann, J.R. (2011). Algae biofuel from wastewater treatment high rate algal ponds. Water Science and Technology, 63 (4), 660-665.
11. De-Bashan, L.E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresource Technology, 101(6), 1611–1627.
12. De-Bashan, L.E., Moreno, M., Herna´ndez, J.P., & Bashan, Y. (2002). Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae Chlorella vulgaris coimmobilized in alginate beads with the microalgae growth-promoting bacterium Azospirillum brasiliense. Water Research, 36, 2941–2948.
13. Delplanque, M., Collet, S., Del Gratta, F., Schnuriger, B., Gaucher, R., and Robinson, B. (2013). Bert Combustion of Salix used for phytoextraction: The fate of metals and viability of the processes. Biomass and Bioenergy, 49, 160-170.
14. Demirbas, A. (2010). Use of algae as biofuel sources. Energy Conversion and Management, 51, 2738–2749.
15. Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M.N.A., & Sampathkumar, P. (2018). Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity. Waste Biomass Valor, 9, 793–800.
16. Elarroussi, H., Elmernissi, N., Benhima, R., El-Kadmiri, I.M., Bendaou, N., Smouni, A., & Wahby, I. (2016). Microalgae polysaccharides a promising plant growth biostimulant. Journal of Algal Biomass Utilization, 7 (4), 55-63.
17. El-Sheekh, M.M., Gharieb, M.M., & Abou-El-Souod, G.W. (2009). Biodegradation of dyes by some green algae and cyanobacteria. International Biodeterioration & Biodegradation, 63, 699–704.
18. Eshetu, G., Tulu D., Endalkachew, W.M., Alemu, L., & Jessica, D. (2018). Response of Kale (Brassica Oleracea L) Crop to Cyanobacterial Biofertilizer in Ziway Area, Ethiopia. Journal of Biology, Agriculture and Health care, 8 (13), 2224-3208. Online at www.iiste.org.
19. Fekry, M.G., EL-Sayed, M.M., Mohamed, S.A.EL-F., Abd ELGawad, Y.EL-S., & Naeem, M.E.D. (2018). The Use of Microalgae in Bioremediation of the Textile Wastewater Effluent. Natural Scince, 16(3), 98-104.
20. Garcia, J., Mujeriego, R., & Hernandez-Marine, M. (2000). High rate algal pond operating strategies for urban wastewater nitrogen removal. Journal of Applied Phycology, 12(3), 331-339.
21. Garcia-Gonzalez, J., & Sommerfeld, M. (2015). Biofertilizer and biostimulant properties of the microalga Acutodesmus dimorphus. Journal of Applied Phycology, 28 (2), 1051-1061.
22. Garg, S.K., and Tripathi, M. (2017). Microbial strategies for discoloration and detoxification of Azo Dyes from textile effluents. Research Journal of Microbiology, 12(1), 1-19.
23. Gavrilescu, M., & Chisti, Y. (2005). Biotechnology- a sustainable alternative for chemical industry. Biotechnology Advances, 23(7-8), 471-499.
24. Grobbelaar, J.U. (2004). Algal nutrition. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Blackwell.
25. Gupta, S.K., Ansari, F.A., Shriwastav, A., Sahoo, N.K., Rawat, I., & Bux, F. (2016). Dual role of Chlorella sorokiniana and Scenedesmus obliquus for comprehensive wastewater treatment and biomass production for bio-fuels. Journal of cleaner production, 115, 255–264.
26. Gupta, V., Prasanna, R., Srivastava, A.K., Sharma, J. (2012). Purification and characterization of a novel antifungal endo-type chitosanase from Anabaena fertilissima. Ann Microbiol, 62, 1089-1092.

27. Hameed, M.S.A. (2006). Continuous removal and recovery of lead by alginate beads, free and alginate-immobilized Chlorella vulgaris. African Journal of Biotechnology, 5 (19), 1819-1823.

28. Hammouda, O., Abdel-Raouf, N., Shaaban, M., & Kamal, M. (2015). Treatment of Mixed Domestic-industrial Wastewater Using microalgae Chlorella sp. Journal of Am Science, 11(12), 303-315.

29. Hannon, M., Gimpel, J., Tran, M., Rasala, B., & Mayfield, S. (2010). Biofuels from algae: challenges and potential. Biofuels, 1,763–784. doi: 10.4155/bfs.10.44. [PMC free article] [PubMed] [CrossRef] [Google Scholar].

30. Hoffmann, J.P. (1998). Wastewater treatment with suspended and nonsuspended algae. Journal of Phycology, 34, 757-763.

31. Hsueh, H.T., Chu, H., & Yu, S.T. (2007). A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. Chemosphere, 66(5), 878-886.

32. Huang, C.H., and Tan, C.S. (2014). A Review: CO2 Utilization. Aerosol and Air Quality Research, 14, 480–499.

33. IEA. (2009). World energy outlook. International Energy Agency, Paris.

34. Jacob-Lopes, E., Lacerda, L.M.C.F., & Franco, T.T. (2008). Biomass production and carbon dioxide fixation by Aphanothece Microscopic Nageli in a bubble column photobioreactor. Biochemical Engineering Journal, 40, 27-34.

35. Jimenez-Perez, M.V., Sanchez-Castillo, P., Romerab, O., Fernandez-Moreno, D., and Perez-Martınez, C. (2004). Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. Enzyme Microbiology Technology 34, 392–398.

36. Kishore J Patil, Harshada Nandre, R T Mahajan, Chiranjib Banerjee, Pratyoosh Shukla and Rajib Bandopadhyay. Plant growth stimulating activity of fresh water micro algae. International Journal of Advanced Research (2015), 3(5), 284-288.

37. Kumar, G., Srivastava, R., & Singh, R. (2013). Exploring biodiesel: chemistry, biochemistry, and microalgal source. International Journal of Green Energy, 10, 775–96.

38. Kurade, M.B., Waghmode, T.R., Kagalkar, A.N., & Govindwar, S.P. (2012). Decolorizations of textile industry effluent containing disperse dye Scarlet RR by a newly developed bacterial-yeast consortium BL-GG. Chemical Engineering Journal, 184, 33–41.

39. Lei, A.P., Hu, Z.L., Wong, Y.S., & Tam, N.F.Y. (2007). Removal of fluoranthene and pyrene by different

40. microalgal species. Bioresource Technology, 98 (2), 273–80.

41. Lim, S.L., Chu, W.L., & Phang, S.M. (2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. Bioresource Technology, 101(19), 7314-22. https://doi: 10.1016/j.biortech.2010.04.092.

42. Lim, S.L., Chu, W.L., & Phang, S.M. (2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. Journal of Bioresource Technology, 101, 7314-7322.

43. Luan, T.G., Jin, J., Chan, S.M.N., Wong, S.Y., & Tam, N.F.Y. (2006). Biosorption and biodegradation of tributyltin (TBT) by alginate immobilized Chlorella vulgrais beads in several treatment cycles. Process Biochemistry, 41(7), 1560–1565.

44. Mallick, N. (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals, 15, 377–390.

45. Manjunath, M., Prasanna, R., Lata, P.D., Singh, R., Kumar, A., Jaggi, S. and Kaushik, B.D. (2010). Bio control potential of cyanobacterial metabolites against damping off disease caused by Pythium aphanidermatum in solanaceous vegetables. Archives of Phytopathology and Plant Protection, 43(7),666- 677.

46. Mantzavinos, D., & Psillakis, E. (2004). Enhancement of biodegradability of industrial wastewaters by chemical oxidation pretreatment. Journal of Chemical Technology and Biotechnology, 79, 431–454.

47. Markou,G., Angelidaki, I., Nerantzis,E., and Georgakakis, D.(2013). Bioethanol Production by Carbohydrate-Enriched Biomass of Arthrospira (Spirulina) platensis. Energies, 6, 3937-3950.

48. Marques, A. E., Miranda, J. R., Batista, A. P., and Gouveia, L. (2011). Microalgae Biotechnological Applications: Nutrition, Health and Environment, Nova Science Publishers, Inc.

49. Megharaj, M., Kantachote, D., Singleton, I., & Naidu, R. (2000). Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal transformation of DDT. Environmental Pollution, 109, 35–42.

50. Mostafa, S.S.M., Shalaby, E.A., & Mahmoud, G.I. (2012) Cultivating Microalgae in Domestic Wastewater for Biodiesel Production. Natural Scientia Biologicae, 4(1), 56-65.

51. Mulbry, W., Kondrad, S., Pizarro, C., & Kebede-Westhead, E. (2008). Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Bioresource Technology, 99, 8137–8142.

52. Mustafa, E.M., Phang, S.M., & Chu, W.L. (2012). Use of an algal consortium of five algae in the treatment of landfill leachate using the high-rate algal pond system. Journal of Applied Phycology, 24, 953-963.

53. Nanjappan, K., Radha, P., Lata, N., & Brahama, D.K. (2007). Evaluation the potential of plant growth promoting cyanobacterial as inoculants for wheat. European Journal of Soil Biology, 43, 23-30.

54. Natarajan, C., Prasanna, R., Gupta, V., Dureja, P. and Nain, L. (2012). Dissecting the fungicidal activity of Calothrix elenkinii using chemical analyses and microscopy. Applied Biochemistry and Microbiology, 48, 51-57.

55. Negara, B.F.S.P., Purnama, D., Angelessy, Z., & Kusuma, A.B (2017). Biogas from Microalgae: A New Perspective of Renewable Energy. International Journal of Research in Advent Technology, 5(8), 2321-9637.

56. Ono, E., & Cuello, J.L. (2006). Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. Biosystems Engineering, 95(4), 597-606.

57. Pacheco, M.M., Hoeltz, M., Morase, M.S.A., and Schneider, R.C.S. (2015a). Microalgae Cultivation techniques and wastewater phycoremediation. Journal of Environmental Science and Health, 50, 573–589.

58. Perazzoli, S., Steinmetz, R.L.R., Mezzari, M.P., Nunes, E.O., & Da Silva, M.L.B. (2013). Biogas production from microalgae biomass. III simpósio internacional sobre gerenciamento de resíduos agropecuários e agroindustriais 12 a 14 de março são pedro – sp.

59. Petrovicˇ,A., & Simonicˇ, M. (2016). Removal of heavy metal ions from drinking water by alginate-immobilised Chlorella sorokiniana. International Journal of Environmental Science and Technology, 13, 1761–1780.

60. Phang, S.M., Chui, Y.Y., Kumaran, G., Jeyaratnam, S., & Hashim, M.A. (2001). High rate algal ponds for treatment of wastewater: a case study for the rubber industry. In: Kojima H, Lee YK, eds. Photosynthetic microorganisms in environmental biotechnology. Hong Kong: Springer-Verlag, 51-76.

61. Phang, S.M., Miah, M.S., Yeoh, B.G., & Hashim, MA. (2000). Spirulina cultivation in digested sago starch factory wastewater. Journal of Applied Phycology, 12(3-5), 395–400.

62. Prasanna, R., Chaudhary, V., Gupta, V., Babu, S., Kumar,A., Singh, R., Shivay, Y.S .and Nain, L. (2013a). Cyanobacteria mediated plant growth promotion and bioprotection against Fusarium wilt in tomato. Europian Journal of Plant Pathology, 136,337-353.

63. Rao, P., Saisha, V., and Bhavikatti, S.S. (2013). Removal of Chromium (VI) from Synthetic Waste water using Immobilized Algae. International Journal of Current Engineering and Technology, 2277 – 4106.

64. Rawat, I., Kumar, R.R., Mutanda, T., & Bux, F. (2010). Dual role of microalgae: Pycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy, 88, 3411-3424.

65. Ruiz-Marin, A., Mendoza-Espinosa, L., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology, 101(1), 58-64.

66. Shanab, S., Ashraf, E., and Shalaby, E. (2012). Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates). Plant Signaling & Behavior, 7(3), 392-399. DOI: 10.4161/psb.19173.

67. Shi, J., Podola, B., & Melkonian, M. (2007). Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. Journal of Applied Phycology. DOI 10.1007/s10811-006-9148-1.

68. Sholkamy, E.N., El-Komy, H., Al-Arfaj, A.A., Abdel-Megeed, A., & Mostafa, A.A. (2012). Potential role of Nostoc muscorum and Nostoc rivulare as biofertilizers for the enhancement of maize growth under different doses of n-fertilizer. Africa Journal Microbiology Research, 6(48), 7435-7448.

69. Sivakami, R., Mahalakshmi, M., and Premkishore, G. (2015). Removal of heavy metals by Biosorption using Cyanobacteria isolated from Freshwater pond. International Journal of current microbiology and applied science, 4(12), 655-660.

70. Smith, VH. (2003). Eutrophication of freshwater and coastal marine ecosystems. Environmental Science Pollution Research, 10, 126-139.

71. Spolaore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 102, 87-96.

72. Staritz, C., Plank, L., and Morris, M. (2016). Global value chains, industrial policy, and sustainable development–Ethiopia's apparel export sector. International centre for trade and development, Geneva.

73. Tam, N.F.Y., Lau, P.S., & Wong, Y.S. (1994). Wastewater inorganic N and P removal by immobilized Chlorella vulgaris. Water Science Technology, 30, 369–374.

74. Valdez, C., Perengüez, Y., Mátyás, B., & Guevara, M.F (2018). Analysis of removal of cadmium by action of immobilized Chlorella sp. micro-algae in alginate beads [version 1; peer review: 2 approved]. F1000 Research 2018, 7, 54.

75. Vannini, C., Domingo, G., Marsoni, M., De Mattia, F., Labra, M., castiglioni, S., & Bracale, M. (2011). Effects of a complex mixture of therapeutic drugs on unicellular algae Pseudokirchneriella subcapitata. Aquatic Toxicology, 101, 459-465.
76. Verma, N.M., Mehrotra, S., Shukla, A., & Mishra, B.N. (2010). Prospective of biodiesel production utilizing microalgae as the cell factories: A comprehensive discussion. African Journal of Biotechnology, 9(10), 1402-11.
77. Zhang, E., Wang, B., Ning, S., Sun, H., Yang, B., Jin, M., & Hou, L. (2012). Ammonia-nitrogen and orthophosphate removal by immobilized Chlorella sp. isolated from municipal wastewater for potential use in tertiary treatment. African Journal of Biotechnology, 11, 6529-6534.
78. Zhang, E., Wang, B., Wang, Q., Zhang, S., & Zhao, B. (2008). Ammonia–nitrogen and orthophosphate removal by immobilized Scenedesmus sp. isolated from municipal wastewater for potential use in tertiary treatment. Bioresource. Technology, 99, 3787–3793.